ANTIBIOTICS AND OTHER NATURALLY OCCURRING HYDROXAMIC ACIDS AND HYDROXAMATES

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ABSTRACT

Naturally occurring hydroxamic acids and hydroxamates are reviewed and classified according to the chemistry of the hydroxamic acid functions. The identity of the Fe(III) trihydroxamate albomycin δ₂ with antibiotic Ro 5-2667 is established.

TABLE OF CONTENTS

1. Monohydroxamic acids
   1.1. Linear hydroxamic acids
       1.1.1. Primary hydroxamic acids
       1.1.2. Secondary hydroxamic acids
   1.2. Cyclic hydroxamic acids
       1.2.1. Pyrazine-type skeleton
       1.2.2. Oxazine-type skeleton

2. Dihydroxamic acids
   2.1. Two linear hydroxamic acid functions
       2.1.1. Head-to-tail connection via ester bond
HUBERT MAEHR

2.1.2. Head-to-head connection via amide bond

\[ \text{NH} \text{--} \text{CO} \]

2.2. Two cyclic hydroxamic acid functions

2.3. Linear and cyclic hydroxamic acid functions

3. Trihydroxamic acids

3.1. Three linear hydroxamic acid functions

3.1.1. Head-to-tail connections

3.1.1.1. Connections via ester bonds

\[ \text{O} \text{--} \text{CO} \text{--} \text{O} \text{--} \text{CO} \]

3.1.1.2. Connections via amide bonds

\[ \text{NH} \text{--} \text{CO} \text{--} \text{NH} \text{--} \text{CO} \]

3.1.2. Head-to-head connections via amide bonds

\[ \begin{array}{c}
\text{NH} - \text{CO} - \text{NH} - \text{CO} \\
\text{HONH} \\
\end{array} \]

3.1.3. Head-to-head connection via amide bonds and head-to-tail connection via ester bond

\[ \text{NH} \text{--} \text{CO} \text{--} \text{O} \text{--} \text{CO} \]

3.2. Three hydroxamic acid functions in a cyclic skeleton

3.2.1. Head-to-tail connections via ester bonds

\[ \text{OC} \text{--} \text{O} \text{--} \text{OC} \]

3.2.2. Head-to-tail connections via amide bonds

\[ \begin{array}{c}
\text{CONH} \\
\text{HNOC} \\
\end{array} \]
NATURALLY OCCURRING HYDROXAMIC ACIDS AND HYDROXAMATES

Hydroxamic acid containing compounds are ubiquitous and frequently bewildering in structural variety and biological implications. Although details of their physiological significance in nature are yet largely unknown, hydroxamic acid moieties are constituents of antibiotics, growth factors, tumour inhibitors, cell-division factors and pigments, and are intimately associated with iron-transport phenomena. Various general and specific aspects of hydroxamic acids have been reviewed previously, but intensified research interest due to the apparent connection of biological activities with hydroxamic acid functions has resulted in the discovery of an ever-increasing number of hydroxamic acid containing compounds. Hence a survey of this class of substances within the framework of a detailed chemical classification is of interest.

Hydroxamic acids may be regarded as derivatives of both hydroxylamines and carboxylic acids. The hydroxylamine part of the more complex hydroxamic acids is almost exclusively derived from hydroxylamino acids or their derivatives: the acyl portion is usually simple and is often acetyl or originates biogenetically from acetate. Classifying the natural hydroxamic acids according to the number of hydroxamic acid units per molecule differentiates three major classes with one, two or three units per molecule. Subdivisions are provided by distinguishing between primary (I) and secondary hydroxamic acids (II) which may be abbreviated by the symbols IA—IIIA. Secondary hydroxamic acids may be linear (II, IIA) or cyclic (III, IIIA). Di- and trihydroxamic acids are further differentiated according to the types of linkages connecting the hydroxamic acid moieties and according to the relative orientation of the hydroxamic acid functions with respect to each other. If the hydroxylamine part of a given hydroxamic acid is designated as the head and the acyl part as the tail (cf. IA—IIIA), one can distinguish between head to tail and head to head connections of two hydroxamic acid functions. Compounds with more than three hydroxamic acid functions per molecule, as well as tail to tail connected hydroxamic acids, have not as yet been reported.

A striking feature of hydroxamic acids is the ability to form coordination compounds. As a bidentate group, the anionic hydroxamate function resembles the acetylacetonato ligand in its behaviour towards various chelate ions. The fact that the corresponding chelate compounds contain
five (IV) and six (V) membered chelate rings obviously is not reflected in the observed stability data.

The monohydroxamato ligand exhibits little cation selectivity with the exception of a distinct preference for Fe(III). Acethydroxamic acid, for example, forms a 3:1 chelate with ferric ions which is more stable than the corresponding Al and Yb complexes by factors of $10^7$ and $10^{12}$, respectively. As complex formation is a pH dependent process, protons effectively compete with chelate ions at low pH. The stepwise destruction of Fe(III) complexes is accompanied by bathochromic shifts and hypsochromic effects in the visible light absorption spectra. The preference for Fe(III) is more pronounced in trihydroxamic acids where three hydroxamate groups create a sexadentate ligand. The corresponding chelations with ferric ions proceed with favourable entropy changes giving rise to positive chelate effects. Accordingly, the Fe(III) complex of the linear trihydroxamic acid ferrioxamine B is 200 times as stable as the tris-acethydroxamato complex.

Utilizing this difference between relative stabilities, Fe(III) chelates of trihydroxamic acids can be distinguished spectroscopically from mono- and dihydroxamic acids. The 1:1 complex of a trihydroxamic acid is stable at pH 2.5 and hence will exhibit the same visible light absorption spectrum at pH 2.5 and 7. In contrast, a tris-monohydroxamato complex at pH 2.5 is extensively dissociated and can be recognized by bathochromic and hypsochromic changes in the spectrum.

Hydroxamate functions, as unsymmetrical bidentate ligands, give rise to hexa-coordinated Fe(III) complexes with octahedral configurations: thus, three molecules of a monohydroxamic acid can be arranged to form four isomeric structures. As represented in Figure 1, structures A and C are enantiomers as are B and D. The A-C pair is the one with the greater degree of order: all nitrogen atoms are equidistant from each other, as are the carbon atoms. Both members of this pair are readily recognized by their facial orientation of those corners of the 5-membered chelate rings which contain either nitrogen (second smallest atoms) or carbon (smallest atoms).

As a result of specific Fe(III) selectivity it is not surprising to find hydroxamic acids, particularly trihydroxamic acids, in association with ferric ions. This distinct preference of Fe(III) masks the affinity of hydroxamate ligands toward other bio-active cations and may thus account for the widespread distribution and accumulation of hydroxamic acids in microbial cells. The low order of selectivity towards Fe(II), however, explains the function of certain hydroxamic acids as iron-transporters in microbial systems.
In this report, we attempt to summarize and classify naturally occurring hydroxamic acids according to structural features previously elaborated. Comparative chemistry should illustrate structure-activity relationships, and brief outlines of pertinent degradation reactions will demonstrate progress in a complex and fascinating field of natural products.

1. MONOHYDROXAMIC ACIDS

1.1. Linear monohydroxamic acids

1.1.1. Primary hydroxamic acids

Simple hydroxamic acids. The simplest hydroxamic acids have been prepared enzymatically. Incubation of acetate with ATP and hydroxylamine in fresh pigeon liver gave acethydroxamic acid; higher fatty acid homologues could be prepared by lipase-catalysed condensations in various liver extracts. Cysteine-activated papain catalysed the replacement of the amide amino-group of a number of amino acid derivatives by hydroxylamine. Similar transamidations could be achieved by cell-free extracts of *Proteus vulgaris* with asparagine and glutamine.

*Actinonin.* The antibiotic actinonin, produced by a *Streptomyces* species and active against a number of Gram positive and Gram negative bacteria, mycobacteria and phages, represents a more complex member of this group. Acid hydrolysis of actinonin yielded L-prolinol, L-valine and D-α-α-n-pentyl succinic acid: Lossen rearrangement of the O-acetyl derivative VI followed by hydrolysis yielded compound VII. Actinonin could thus be formulated as VIII.

![Figure 1. Isomeric structures of a Fe(III) trihydroxamate.](image-url)
1.1.2. Secondary hydroxamic acids

**Hadacidin.** Produced by several *Penicillium* species, hadacidin (X) is active as tumour inhibitor and plant growth retardant. It was identified by hydrolysis yielding formic acid and hydroxylaminoacetic acid and synthesized\(^{16}\) by formylation of hydroxylaminoacetic acid, which in turn was prepared from ethyl acetoacetate and nitric oxide. A more recent synthesis\(^{17}\) involved N-alkylation of *anti*-benzaldoxime\(^{18-19}\) to give N-(carboxymethyl)-α-phenyl nitrone (IX) which was decomposed and formylated to yield N-formylhydroxyamino acetic acid, X.

\[
\begin{align*}
\text{O} & \quad \text{H} & \quad \text{N} & \quad \text{COOH} \\
\text{H} & \quad \text{N} & \quad \text{COOH} \\
\text{CH}_2\text{OH} & \quad \text{AC}_2\text{O} & \quad \text{HCOOH} & \quad \text{CHO}
\end{align*}
\]

\((\text{IX})\) \hspace{1cm} \text{Hadacidin} \hspace{1cm} \text{(X)}

**Fusarines.** Although *Fusarium* species produce *cis*-fusarinine\(^\dagger\) in abundance, this compound is without apparent growth factor or antibiotic properties\(^{10}\). Its presence in nature is significant in that it constitutes the monomeric unit of fusarinines A and B\(^{20}\) and fusigen\(^{21}\) and is contained in ferrirhodin\(^{22}\). *cis*-Fusarinate is one of the simple representatives containing \(N^5\)-hydroxyornithine, XII, a hydroxylamine derivative frequently found in natural trihydroxamic acids. \(N^5\)-hydroxyornithine was originally described as a constituent of ferrichrome\(^{23}\) and was synthesized in eight steps from glutamic acid XVI A via the corresponding nitro- and hydroxylamino-hydantoin in 7 per cent yield\(^{24}\). An improved synthesis via nitrone XVI C was achieved in our laboratory with 40 per cent yield, starting with 5-hydroxy-2-aminovaleric acid XVI B\(^{25}\). Treatment of XII with methanolic hydrogen chloride afforded the hydrochloride of 1-hydroxy-3-amino-2-piperidone, XV\(^{26}\).

\(\dagger\) This compound was originally called fusarinine, but should be termed *cis*-fusarinine to differentiate it from 3-butylypyridine, the decarboxylation product of fusaric acid, which is also named fusarinine (cf. Pl. A. Plattner, W. Keller and A. Boller. *Helv. Chim. Acta* 37, 1379, 1954) and from the recently discovered *trans*-fusarinine (cf. ref. 10b).
NATURALLY OCCURRING HYDROXAMIC ACIDS AND HYDROXAMATES

Structure determination\textsuperscript{10a} of cis-fusarinine (XI) is primarily based on degradation reactions summarized below. Reductive hydrolysis with 47 per cent hydriodic acid gave ornithine, XIII, whereas 6 N hydrochloric acid hydrolysis yielded $N^5$-hydroxyornithine, XII. 3-Methylpent-2-eno-5-lactone (XIV) was obtained upon periodic acid oxidation\textsuperscript{27}. Lactone XIV, which can be regarded as $\Delta^2$-anhydromevalonic acid lactone, had previously been identified as a metabolite of various fungi\textsuperscript{28a}.

trans-5-Hydroxy-3-methylpent-2-enoic acid is found in substrates of various moulds\textsuperscript{28b}; the geometric isomer of fusarinine, trans-fusarinine, has recently been described as a naturally occurring product\textsuperscript{10b} and constituent of more complex compounds containing hydroxamate functions.

1.2. Cyclic hydroxamic acids

1.2.1. Pyrazine-type skeleton

\textit{Aspergillic acid}. One of the first antimicrobial mould metabolites described is aspergillic acid\textsuperscript{29} with activity against certain Gram positive and many Gram negative bacteria and known as a microbial growth factor\textsuperscript{30, 31}. It is presumably identical with an antibiotic isolated by Glistere\textsuperscript{32, 33}. 

609
Aspergillic acid, elaborated by *Aspergillus flavus*, was recognized as a pyrazine cyclic hydroxamic acid\textsuperscript{34}, the first example of a naturally occurring hydroxamic acid. Aspergillic acid exhibits weakly acidic and basic properties and gives a wine-red colour with ferric chloride. Reduction with either hydriodic acid in acetic acid or with hydrazine, yielded deoxyaspergillic acid, 3,6-dialkyl-2(1H)-pyrazinone, to which tentative structure XIX was assigned. Bromine oxidation of both aspergillic and deoxyaspergillic acids, followed by reduction, gave a diketopiperazine which did not depress the melting point of isoleucine anhydride, XXII. Aspergillic acid was therefore thought to be XXV or the tautomeric hydroxamic acid\textsuperscript{35}.

\begin{align*}
\text{Flavacol} & \quad \text{(XVII)} \\
\text{Deoxyaspergillic acid} & \quad \text{(XVIII)} \\
\text{Leu anhydride} & \quad \text{(XX)} \\
\text{Leu-isoleu anhydride} & \quad \text{(XXI)} \\
\text{Isoleu anhydride} & \quad \text{(XXII)} \\
\text{Neoaspergillic acid} & \quad \text{(XXIII)} \\
\text{Aspergillic acid} & \quad \text{(XXIV)} \\
\end{align*}

A depression of a mixture melting point\textsuperscript{36} of the synthetic racemate of XIX\textsuperscript{37} by racemized deoxyaspergillic acid, however, indicated that the two compounds were different. Hydrolysis of the diketopiperazine derived from aspergillic acid indeed yielded leucine and isoleucine and could thus be formulated more correctly as XXI\textsuperscript{38}.

To distinguish between the two possible structures for deoxyaspergillic acid, racemic XVIII was synthesized and found to be identical with racemized deoxyaspergillic acid. This established the structure of aspergillic acid as...
NATURALLY OCCURRING HYDROXAMIC ACIDS AND HYDROXAMATES

6-sec-butyl-1-hydroxy-3-isobutyl-2(1H)pyrazinone or its tautomer XXIV, 6-sec-butyl-3-isobutyl-2-pyrazinol-1-oxide\textsuperscript{39}; racemic aspergillic acid was ultimately synthesized\textsuperscript{40}.

Granegillin. Isomeric with aspergillic acid, granegillin is also produced by Aspergillus flavus but differs from aspergillic acid in several chemical and biological aspects\textsuperscript{41}. No structural formula has been proposed for this antibiotic.

Neoaspergillic acid. Another isomeric aspergillic acid, neoaspergillic acid, is elaborated by Aspergillus sclerotium\textsuperscript{42}. The close chemical relationship to aspergillic acid is reflected in very similar biopotencies. The structure of neoaspergillic acid (XXIII) was deduced from the great similarity to aspergillic acid and n.m.r. spectroscopy and was proved by reduction to pyrazinone XV\textsuperscript{143} and synthesis\textsuperscript{40}. Interestingly, compound XVII was previously found with aspergillic acid in culture filtrates of Aspergillus flavus and was termed flavacol\textsuperscript{44}, although it would now, more appropriately, be regarded as deoxyneoaspergillic acid.

Hydroxyaspergillic acid. Shortly after the discovery of aspergillic acid, it was observed that Aspergillus flavus, under modified fermentation conditions, produced a new antibiotic\textsuperscript{33}. This substance was named hydroxyaspergillic acid\textsuperscript{45}.

Treatment of hydroxyaspergillic acid with hydriodic acid in phosphoric acid afforded racemic deoxyaspergillic acid XVIII and established the close relationship to aspergillic acid. Hydriodic acid in acetic acid, however, yielded a deoxyaspergillic acid (XXVII) with an additional double bond in conjugation with the pyrazinone ring. Cleavage of XXVII with ozone gave optically inactive XXVIII and acetaldehyde, confining the hydroxyl groups to the sec-butyl side chain. The assignment of a tertiary hydroxyl group is supported by the observed reluctance towards esterification and a negative iodoform reaction, establishing the structure of hydroxyaspergillic acid as XXVI.

Neohydroxyaspergillic acid. The second example of a hydroxyaspergillic acid...
HUBERT MAEH

acid was provided by Aspergillus sclerotiorum which produced an antibiotic with antiphage and antibacterial activity. The compound was identified as a hydroxyaspergillic acid and was termed neo hydroxyaspergillic acid. Several years later the same compound was reported to be co-produced with aspergillic acid by the same mould. Hydroxy- and neo hydroxyaspergillic acids have similar biological activities but are less active antibiotics than aspergillic and neo aspergillic acids. Assignment of probable structure XXIX to neo hydroxyaspergillic acid is based on biogenetic considerations as well as on n.m.r. spectra, whose interpretations were facilitated by the availability of previously defined aspergillic acids.

\[
\text{Hydroxyaspergillic acid} \quad (\text{XXVI})
\]

\[
\text{Neohydroxyaspergillic acid} \quad (\text{XXIX})
\]

\[
\text{Mutaaspergillic acid} \quad (\text{XXX})
\]

Mutaaspergillic acid. A third variation of hydroxylated aspergillic acids, muta aspergillic acid, was obtained from a culture of Aspergillus oryzae. Guided by the investigations of Dutcher on hydroxyaspergillic acid, hydriodic acid in phosphoric acid treatment yielded dideoxymuta aspergillic acid, 3-isobutyl-6-isopropyl-2(1H)-pyrazinone, whose constitution was confirmed by synthesis and, subsequently, by degradation to valine and leucine following the example of previous studies. The side chain hydroxyl group was assigned to the tertiary position of the isopropyl chain in view of optical inactivity of muta aspergillic acid, a negative iodoform reaction and the reluctance to form a dehydro compound which could be obtained from hydroxyaspergillic acid. Structure XXX for muta aspergillic acid was also confirmed by synthesis.

1.2.2. Oxazine-type skeleton

2,4-Dihydroxy-1,4-benzoazin-3-one; 2,4-dihydroxy-7-methoxy-1,4-benzoazin-3-one and the two corresponding glucosides. The immediate precursors of benzoazoline-2-one (BOA, XXXI) and 6-methoxybenzoazoline-2-one (MBOA, XXXII) found in rye, wheat and maize plants were identified as two cyclic hydroxamic acids, 2,4-dihydroxy-1,4-benzoazin-3-one XXXIII and the corresponding 7-methoxy derivative XXXIV. The latter two compounds are derived enzymatically from the corresponding glucosides XXXV and XXXVI.

\[
R = \text{H (BOA) (XXXI)}
\]

\[
R = \text{CH}_3\text{O (MBOA) (XXXII)}
\]
BOA is found in crushed rye plants, whereas MBOA originates from maize plants. There is strong evidence that neither of the two compounds is present in uninjured tissue. Thus, apparently stable hydroxamic acids occurring in living plants decompose upon plant injury to yield substances with modified biological activity. In contrast to the glucosides and hydroxamic acids, the corresponding aglucones as well as BOA and BMOA exhibit growth-inhibiting properties with pronounced fungistatic activities. Whereas the decomposition of the glucosides to the corresponding aglucones occurs enzymatically, the ring contraction appears to be a relatively simple process and occurs readily within the pH range 4—8. Proof for the structure of glucoside and aglucone was ascertained by the reactions summarized below. O-amino-phenol accompanied by hydroxylamine or glycine, depending upon the reaction conditions, was obtained from compounds and . Hydrogenolysis yielded and which no longer gave positive tests with ferric chloride. As expected, could not be converted to benzoxazolinone upon treatment with hot water. The structures of aglucone and its reduction product, , were confirmed by syntheses.

2. DIHYDROXAMIC ACIDS

2.1. Compounds with two linear hydroxamic acid functions

2.1.1. Hydroxamic acid functions connected head to tail via ester bond

Fusarimine A. A depsipeptide composed of two cis-fusarinine units was obtained from a Fusarium roseum culture and termed Fusarimine A, . Hydrolysis of one mole of fusarimine A in water cleaved the ester bond selectively giving rise to two moles of cis-fusarinine, XI. Dilute acid, protonating the free amino group and thus protecting the vicinal ester linkage against hydrolytic cleavage, liberated 3-methylpent-2-eno-5-lactone, XIV. Periodic acid oxidation cleaved both hydroxamic acid functions selectively and resulted in lactone XIV: the incipient nitrogen-bearing terminals combined to the dimeric nitroso compound which, upon hydrogenolysis, yielded ornithine XIII and 5-O-(L-ornithyl)-DL-3-methylpentanoic acid, XLI. The structure of XLI was confirmed by synthesis, providing definite proof for the existence of the ester linkage in fusarimine A.

2.1.2. Hydroxamic acid functions connected head to head via amide bonds

Rhodotorulic acid. This compound was isolated from iron-deficient cultures of a red yeast, Rhodotorula pilimanae, and other organisms and showed potent growth-stimulating activity for Arthrobacter species. Rhodotorulic acid was identified as the diketopiperazine of N-acetyl-L-N-hydroxynitrite, XLII, and is thus chemically related to sideramines of the ferrichrome type, but does not show the antagonism against albomycin, which is characteristic of the sideramine growth factors.
Hydrolyses of rhodotorulic acid with hydrochloric acid and hydriodic acid yielded XII and XIII, respectively, whereas hydrogenolysis\textsuperscript{59} afforded \(N^5\)-acetyl-L-ornithine anhydride, identical with a synthetic specimen.

\textbf{Dimeric acid.} Closely related to rhodotorulic acid, dimeric acid, XLIII, is produced by \textit{Fusarium dimerum} and represents the diketopiperazine of trans-fusaridine, \(N^5\)-(trans-5-hydroxy-3-methylpent-2-enoyl)-\(N^5\)-hydroxyornithine\textsuperscript{60,10b}. In contrast to rhodotorulic acid, dimeric acid is reported to antagonize the action of ferrimycin and albomycin\textsuperscript{60}.

\[
\text{Rhodotorulic acid: } R = \text{CH}_3 \\
\text{Dimeric acid: } R = \text{CH}_2-\text{CH}_2\text{OH}
\]

\textbf{Aerobactin.} A pentaprotic acid with two hydroxamic acid moieties, produced by \textit{Aerobacter aerogenes} and related strains, to which structure XLIV was assigned\textsuperscript{61}, was named aerobactin. Hydriodic acid hydrolysis yielded L-lysine, whereas hydrochloric acid treatment liberated citric acid and \(N^6\)-hydroxylysine, XLV, an amino acid originally discovered by Snow\textsuperscript{62} as a constituent of mycobactins. The presence of \(N^6\)-acetyl-\(N^6\)-hydroxylysine was indicated by periodate oxidation\textsuperscript{27} yielding acetic acid and the n.m.r. spectrum of aerobactin was in satisfactory agreement with assignment XLIV.

\[
\text{Aerobactin (XLIV)}
\]

\textbf{Ferribactin.} Cultures of \textit{Pseudomonas fluorescens} Migula produce a polypeptide, called ferribactin, which appears to be a dihydroxamic acid\textsuperscript{63}. The hydroxamic acid functions are due to two \(N^5\)-acetyl-\(N^5\)-hydroxyornithine moieties. Since ferribactin forms a 1:1 complex with Fe(III), it was concluded that additional ligands, other than hydroxamates, must be present. The structure of this compound is as yet unknown, a molecular weight between 1200–1300 has been estimated and its listing in this section is tentative.
NATURALLY OCCURRING HYDROXAMIC ACIDS AND HYDROXAMATES

2.2. Compounds with two cyclic hydroxamic acid functions

Puicherrimic acid. Under certain conditions Candida pulcherrima produces a red pigment which was described as an Fe(III) containing polymer, pulcherrimin\(^{64}\). To the iron-free monomer, termed puicherriminic acid\(^{65}\), tentative structure XLVI was assigned\(^{50}\), for reductive pyrolysis of pulcherrimin yielded leucine anhydride, XX. Furthermore, the Fe(III) complex of aspergillic acid resembled pulcherrimin according to visible and ultraviolet light absorption spectra, but there appeared to be two hydroxamic acid functions in pulcherriminic acid as judged from the N:O ratio and the insolubility of pulcherrimin due to the high molecular weight. Pulcherriminic acid was thus regarded as a quadridentate ligand.

Structure XLVI subsequently gained support when the diprotic pulcherriminic acid could be degraded with zinc and acetic acid to give L-leucine anhydride\(^{65}\).

A comparison of pulcherriminic acid\(^{65}\) with synthetic 1,4-dihydroxy-3,6-diisobutyl-2,5-dioxopiperazine, XLVI, however, showed the two compounds to be different and alternative tautomer XLVII was proposed for puicherriminic acid\(^{66}\). Realizing the inability of XLVII to give rise to optically active leucine, MacDonald sought to resolve this discrepancy\(^{67}\).

He isolated pulcherrimin from three different strains of Candida pulcherrima but, although the resulting three samples of pulcherriminic acid were identical, they differed from the previously described\(^{66}\) pulcherriminic acid. Nevertheless, MacDonald’s pulcherriminic acid was reduced to DL-leucine anhydride with zinc and acetic acid and n.m.r. spectra indicated two isobutyl side chains and no ring hydrogen atoms. Thus structure XLVIII, or possibly its tautomer XLVII, was postulated for MacDonald’s pulcherriminic acid. This assignment was supported by analysis of reduction products of pulcherriminic acid\(^{67}\) and by synthesis of 2,5-dihydroxy-3,6-diisobutylpyrazine 1,4-dioxide (XLVIII) and its Fe(III) complex, which proved to be identical with pulcherriminic acid and pulcherrimin, respectively\(^{68}\).

Mycelianamide. Preferentially active against Gram positive bacteria, this weakly acidic, laevorotatory antibiotic is elaborated by Penicillium griseofulvum Dierckx\(^{69}\). Mild hydrolytic conditions led to the optically inactive p-myceloxybenzoic acid (L) and its amide (LI), whereas vigorous acid hydrolysis yielded ammonia, carbon dioxide and aminoacetophenone, LII.
The hydrolytic instability of the ether function of the \( p \)-myceloxybenzoic acid moiety was correctly attributed to an allylic ether\(^6\). In an extension of this work\(^7\), myceliamide was reduced to the ferric chloride negative dideoxymycelianamide LIV which, in turn, yielded alanine LIII and \( p \)-hydroxyphenylpyruvic acid LV upon acid hydrolysis. Alanine was also detected in acid hydrolysates of XLIX. These results suggested a cyclic dihydroxamic acid XLIX, an assignment which was supported by the similarity of the u.v. spectra of myceliamide and the dideoxy compound and by a strong band at 1675 cm\(^{-1}\) in the i.r. spectrum, indicating an unstrained cyclic amide. The correct structure of the mevalonic acid derived \( C_{10}H_{17} \) side chain was established in the course of a reexamination several years later\(^7\). The n.m.r. spectra strongly suggested a geranoxy or neroxy structure which was supported by oxonolysis products LVI, 4-carbamoylphenoxyacetic acid, and LVII, laevulinic acid. The two possible geometrical isomers of \( p \)-myceloxybenzamide were synthesized establishing the structure of LI as \( p \)-geranoxybenzamide and hence the total structure of myceliamide (XLIX). A synthesis of racemic dideoxymycelianamide has been reported recently\(^7\).

2.3. Compounds with linear and cyclic hydroxamic acid functions

*Mycobactins.* The present knowledge of the chemistry of mycobactins is largely due to the contributions of Snow which were summarized recently\(^7\).

Mycobactins, apparently produced exclusively by mycobacteria, are highly mycobacteria-specific growth factors. Mycobactins are chemically closely related; the chemical differences are characteristic for certain groups of the producing microorganism. The basic formula LIX pertains to all mycobactins; the chemical variations are due to different substituents \( R_1 \) to \( R_5 \) and may be further complicated by stereoisomeric possibilities.

Cleaving the ester linkage, mild alkaline treatment of mycobactins yielded the corresponding mycobactic acids LX and cobactins LXI. Further acid degradation of mycobactic acid liberated a substituted benzoic acid of transient existence giving rise to carbon dioxide and LXII, which was either phenol (\( R_2 = H \)) or \( m \)-cresol (\( R_2 = \text{CH}_3 \)), depending upon the mycobactin investigated. In addition to this aromatic fragment the acid hydrolysate of LX always contained a hydroxyamino acid LXIII, which was serine (\( R_3 = H \)) or threonine (\( R_3 = \text{CH}_3 \)), \( N^6 \)-hydroxylysine (XLV) and a more or less complex fatty acid LXIV. The presence of the hydroxyphenyloxazoline nucleus was confirmed by u.v. spectral data and the hydroxamic acid linkage of the fatty acid side chain \( R_1 \) was demonstrated by periodate oxidation, suggesting structure LX for mycobactic acid.

Acid hydrolysis of cobactin LXI again yielded \( N^6 \)-hydroxylysine as well as a hydroxyamino acid LXV, which, in the case of most mycobactins, was either 3-hydroxybutyric acid (\( R_4 = \text{CH}_3 \); \( R_5 = \text{CH}_3 \)) or 3-hydroxy-2-methylpentanoic acid (\( R_4 = \text{C}_2\text{H}_5 \), \( R_5 = \text{CH}_3 \)). Similar to mycobactins and mycobactic acid, cobactin gives a wine-red ferric chloride colour, is not reducing and possesses only one ionizing group (\( \text{PK}_a \) 9.1), indicating that both carboxyl groups as well as the amino group are masked. The resulting assignment LXI for cobactin provides a hydroxyl group which is involved...
in the formation of the ester linkage with the carboxyl group of mycobactinic acid to yield mycobactin, LIX.

Although they are dihydroxamic acids, mycobactins form extremely stable 1:1 complexes with Fe(III) ions, a third bidentate ligand being contributed by the phenolic hydroxyl group and the oxazoline ring nitrogen.

3. TRIOHYDROXAMIC ACIDS

Trihydroxamic acids of natural origin which are able to form stable Fe(III) trihydroxamate complexes and exhibit absorption maxima between
NATURALLY OCCURRING HYDROXAMIC ACIDS AND HYDROXAMATES

420–440 nm, were originally designated as siderochromes\textsuperscript{73,2}. Siderochromes are subdivided according to biological activity into sideromycins, designating siderochromes with antibiotic activity, and sideramines, representing growth-factor siderochromes. More specifically, therefore, the term siderochrome should be limited to natural trihydroxamic acids or their Fe(III) chelates which either lack sideromycin or sideramine activity, which can exhibit both of these activities, or whose biological activities are undetermined\textsuperscript{74–76}.

Sideromycins have been differentiated according to biological activity and paper chromatographic behaviour\textsuperscript{73}. Rapid development of resistant colonies with cross-resistance toward all sideromycins appears to be peculiar to these antibiotics. Antagonism between sideromycins and sideramines is of diagnostic value\textsuperscript{74} and considerable biological significance\textsuperscript{14}. Although sideromycins have as yet only been found as metabolites of streptomycetes, sideramines have been demonstrated in cultures of streptomycetes and other actinomycetes, fungi and bacteria\textsuperscript{74,75}.

3.1. Compounds with three linear hydroxamic acid functions

3.1.1. Hydroxamic acid functions connected head to tail

3.1.1.1. Connection via ester bond—Fusarinine B Total acid hydrolysates of all cis-fusarinines yielded the same product profiles consisting of N\textsuperscript{5}- hydroxyornithine, XII, and 3-methylpent-2-eno-5-lactone, XIV. Brief treatment of fusarinine B in hot water yielded cis-fusarinine (XI) and fusarinine A (XXXIX). On continued hydrolysis cis-fusarinine is the principal degradation product. The trihydroxamic acid nature of fusarinine B was demonstrated by the relative stability of the Fe(III) complex in acidic solution. Closely related to and coproduced with fusarinines A and C, fusarinine B can thus be formulated as LXVI and regarded as a sideramine, for fusarinines A, B and C exhibit slight growth-factor activity with Arthrobacter JG-9\textsuperscript{20}.

\[ \text{LXVI} \]

Fusarinine B

621
3.1.1.2. Connections via amide bonds—Ferrioxamines and ferrimycins. Ferrimycins, metabolites of *Streptomyces griseoflavus*, exhibit pronounced activity against Gram positive microorganisms. Extended efforts to isolate related substances resulted in the discovery of a new class of sideramines, the ferrioxamines, which are chemically related to ferrimycins and were found in a large variety of microorganisms. In spite of the chemical similarity to ferrimycins, ferrioxamines, as a rule, do now show antibiotic activity, but antagonize the antibiotic activity of ferrimycins and other sideromycins against Gram positive organisms.

An acid hydrolysis product, common to both ferrimycins and ferrioxamines, was soon recognized as 1-amino-5-hydroxylaminopentane, LXVII. Ninhydrin-positive and reducing, LXVII is dibasic with pKₐ values 5.30 and 9.80. Hydrogenolysis of LXVII gave 1,5-diaminopentane, LXVIII. In analogy to the work of Snow, LXVIII could be obtained directly from ferrioxamines and ferrimycins as a result of reductive hydrolysis with hydriodic acid. The structure of LXVII was confirmed by synthesis starting with 1-phthalimido-5-bromopentane and later by a reaction sequence starting with 1-benzoyl-5-chloropentane.

Among the ferrioxamines, ferrioxamine B (LXIX) is the most abundant representative. It is basic (pKₐ 8.79) and upon acid hydrolysis yielded acetic acid, succinic acid and 1-amino-5-hydroxylaminopentane in a molar ratio of 1:2:3. To account for the observed stability of the Fe(III) complex, the sequence depicted in structure LXIX was postulated, an assignment later confirmed by synthesis.

N-Acetylfèrrioxamine B occurs in nature and was designated ferrioxamine D₁ (LXX) and nocardamine. The structure of the zwitter-ionic Ferrioxamine 0 (LXXI) was confirmed by conversion to nocardamine and total synthesis.

Diverging from the basic theme, ferrioxamines A₁ and A₂ contain 1-amino-4-hydroxylaminobutane residues. The structure of ferrioxamine A₁, LX XII, was elucidated. Ferrioxamine A₂ differs from A₁ in that it contains two 1-amino-4-hydroxylaminobutane residues of unknown sequence. The structures of the two basic ferrioxamines C and F are still unknown.

Among the ferrimycins, component A₁, LXXIII, was studied most extensively. The iron-binding centre of ferrimycin A₁ is identical to LXIX and LXX. Controlled hydrochloric acid hydrolysis of ferrimycin A₁ gave LXXIV and LXXV in a molar ratio of 2:1. The structure of fragment LXXV was deduced by further degradation reactions yielding LXVII, ammonia, and 3-amino-5-hydroxybenzoic acid, and by n.m.r. studies and mass spectrometry.

3.1.2. Three hydroxamic acid functions connected head to head via amide bonds. Ferrichrome, ferrichrome A and C. Cultures of the smut fungus *Ustilago sphaerogena* produced two microbial growth factors, ferrichrome and the less active ferrichrome A₁.

Two factors, properties of N⁶-hydroxylysine known due to the investiga-
NATURALLY OCCURRING HYDROXAMIC ACIDS AND HYDROXAMATES

\[
\text{Ferrioxamine B: } R = H \quad (LXIX)
\]
\[
\text{Ferrioxamine } D_1: R = \text{COCH}_3 \quad (LXX)
\]

\[
\text{Ferrimycin } A_1 \quad (LXXIII)
\]

\[
\text{(LXVIII)} \quad \text{(LXVII)} \quad \text{(LXXIV)} \quad \text{(LXXV)}
\]

623
tions of Snow and the discovery of the cleavability of hydroxamic acid C—N bonds by periodate, facilitated the elucidation of the iron-binding centres as trihydroxamic acids consisting of three $N^5$-acyl-$N^5$-l-hydroxyornithine moieties. One molecule of ferrichrome contains three molecules of $N^5$-acyl-$N^5$-hydroxyornithine and 3 molecules of glycine. To account for the observed neutrality, a cyclic hexapeptide was proposed whose sequence was determined as depicted in LXXVI. Ferrichrome has now been synthesized, confirming earlier structural assignments.

Ferrichrome A (LXXVII) is an acidic substance and differs from ferrichrome in that two of the glycine moieties are replaced by serine and the acyl portions of the three hydroxamic acid functions are derived from 2-methylpropene-1,3-dicarboxylic acid with the double bond $\alpha$-$\beta$ to the hydroxamate function. The amino acid sequence assigned was confirmed by crystallographic analysis, which also revealed the absolute configuration $C$ (Figure 1) about the Fe(III) ion.

Ferrichrome C resembles ferrichrome. Acid hydrolysis indicated that one of the glycine moieties is replaced by alanine, suggesting structure LXXVIII for ferrichrome C where the hydroxymethyl group of serine is replaced by a methyl group.

Ferricrocin, ferrichrysin, ferrirubin and ferrirhodin. An extended search for sideramines among Aspergillaceae revealed not only the presence of ferrichrome and coprogen but a series of new, ferrichrome-like sideramines. In regard to the amino acid constituents, Ferricrocin, LXXVIII, assumes a position between ferrichrome and ferrichrome A since it contains two molecules of glycine, one of serine and three molecules of $N^5$-acyl-$N^5$-hydroxyornithine. The amino acid sequence is assumed to be analogous to that of ferrichrome A. Ferrichrysin (LXXXIX), ferrirubin (LXXX), and ferrirhodin (LXXXXI) possess the hexapeptide moiety of ferrichrome A: the differences are localized in the acyl portions of the hydroxamate functions. Cleavage of the hydroxamate functions of ferrirhodin by periodate yielded lactone XIV, demonstrating the similarity to cis-fusaridine and...
Ferrichrome (LXXVI)

Ferriocrocin (LXXVIII)

Ferrichrom \( R = \) HOOC-\( \text{H}_3\text{C} \)-

Ferrichrysin \( R = \) CH\(_3\)-

Ferrirubin \( R = \) \(

Ferrirubin \( R = \) \(

Ferrirhodin \( R = \) \(\text{H}_2\text{O} \)-

Ferrirhodin \( R = \) \(\text{H}_2\text{O} \)-
establishing the acyl portions of the hydroxamate groupings as \textit{cis}-5-hydroxy-3-methylpent-2-enoyl. Similarly, ferrirubin gave rise to \textit{trans}-5-hydroxy-3-methylpent-2-enolic acid\textsuperscript{28b} which did not lactonize. Both ferrirrhodin and ferrirubin gave identical hexahydro derivatives upon catalytic hydrogenation confirming structures LXXX and LXXI as geometric isomers. Ferrichrysin possesses the hydroxamate arrangement of ferrichrome (LXXVI) and ferricrocin (LXXVIII).

Mild acid hydrolysis was found to cleave hydroxamic acids to liberate carboxylic acids and hydroxylamine moieties. Reactylation followed by O-deacylation in methanol-ammonia reconstituted hydroxamic acids\textsuperscript{96}. Thus, ferrirubin (LXXX) was converted to ferrichrysin as was ferrichrome A establishing the identity of the hexapeptide moieties involved\textsuperscript{22}.

\textit{Albolymycin, grisein, alveomycin, antibiotics A-1787, LA 5352, LA 5937, 10073, Ro 5-2667, Ro 7-7730 and Ro 7-7731.}

Albolymycin, produced by \textit{Actinomyces subtropicus}\textsuperscript{98}, is an antibiotic with broad-spectrum \textit{in vitro} activity\textsuperscript{3}. Initial separation studies revealed heterogeneity of albomycin preparations\textsuperscript{99}; gradient elution chromatography on a sulphonated polystyrene resin column and electrophoresis demonstrated the presence of one major antibiotic, albomycin \(\delta_2\). Antibiotics \(\delta_1\) and \(\varepsilon\) were recognized as products resulting from degradation of the unstable albomycin \(\delta_3\)\textsuperscript{100}. Albomycin \(\delta_2\) in dilute acid, at room temperature, was completely converted to the basic albomycin \(\varepsilon\); upon heating in neutral, aqueous solution albomycin \(\delta_1\) was the major product\textsuperscript{101}. Curiously, albomycin \(\varepsilon\) at 28° acts as an antibiotic against \textit{B. subtilis}, but at 38° it is a sideramine and antagonizes the activity of albomycins \(\delta_1\) and \(\delta_2\)\textsuperscript{102,103}. The latter are also subject to competitive inhibition by sideramines of the ferrichrome and ferrioxamine types\textsuperscript{7}.

Hydrochloric acid hydrolysis of a mixture of albomycin \(\delta_1\) and \(\delta_2\) was reported to yield serine and ornithine in an equimolar ratio, as deduced by quantitative evaluation of paper chromatograms\textsuperscript{99}. In an independent investigation by Poddubnaya and Krysin\textsuperscript{104}, albomycin was claimed to contain \(N^5\)-hydroxyornithine, serine and iron in molar ratios of 3:3:1, but experimental details were not disclosed.

In the meantime it was demonstrated that hydrochloric acid hydrolysis in the presence of Fe(III) ions was unsuitable for the determination of hydroxylamine moieties but quantitation could be achieved by reductive hydrolysis\textsuperscript{23,75,96}. Alternatively, catalytic reduction of a hydrochloric acid hydrolysate, obtained after removal of Fe(III) ions, yielded reliable identification of the corresponding amines\textsuperscript{22}. Yet, even in view of these findings, the postulates of equimolar amounts of serine and \(N^5\)-hydroxyornithine in albomycin hydrolysates were retained\textsuperscript{103,102}. A cyclic hexapeptide structure was inferred in view of the absence of terminal amino groups and three free serine hydroxyl groupings were postulated on the basis of partial esterification with sulphuric acid\textsuperscript{104}. Three acetyl residues at the \(N^5\)-positions of the \(N^5\)-hydroxyornithine moieties were found to contribute to the Fe(III) trihydroxamate part\textsuperscript{102,104}, providing a structural arrangement resembling sideramines of the ferrichrysin type.

In addition to these structural features, albomycin was disclosed to contain a pyrimidine and one sulphur atom, both attached to the same
serine moiety\textsuperscript{106}. Partial hydrochloric acid hydrolysis reportedly gave compounds which, upon total hydrolysis, yielded 3-methyluracil (LXXXVI) and serine in molar ratios of 1:1, 1:2 and 1:3\textsuperscript{107,108}. Degradative oxidation of the hydroxamic acid functions of albomycin, followed by partial hydrolysis, allegedly gave six peptides whose composition indicated a peptide moiety as depicted in LXXXII-LXXXIV\textsuperscript{108,109}. The same hexapeptides were

![Diagram of albomycin structure](image)

proposed for all albomycins; the differences were assumed to be localized in the pyrimidine moieties\textsuperscript{106,110}. 3-Methyluracil (LXXXVI) was obtained upon hydrochloric acid hydrolysis of a mixture of albomycins\textsuperscript{111}, whereas perchloric acid hydrolysis of albomycins $\varepsilon$ and $\delta_2$ gave 3-methylcytosine (LXXXVII)\textsuperscript{101}. A comparison of the u.v. spectra of 1,3-dimethyluracil (LXXXVII) and 1,3-dimethylcytosine (LXXXIX) with the spectra of albomycins $\delta_1$ and $\varepsilon$ suggested that the 3-methylpyrimidines were linked to the serine moiety via position 1\textsuperscript{106}. A similar conclusion was reached after isolation of 3,N$^4$-dimethylcytosine (XC) from a perchloric acid hydrolysate.
of albomycin e, previously treated with dimethyl sulphate. Diazomethane and dimethyl sulphate failed to methylate the pyrimidine moiety of albomycin \( \delta_2 \). The absence of brominated pyrimidines in albomycins \( \delta_1 \) and \( \delta_2 \), after treatment with bromine in acetic acid, was taken as indication that the albomycins contained 5-substituted pyrimidines. Implying u.v.-spectral similarity between 1,3-dimethyl-\(N^4\)-acetylcytosine (XCI) and albomycin \( \delta_2 \), Sorm and co-workers proposed a 1-substituted 3-methyl-\(N^4\)-acetylcytosine moiety as the chromophore of albomycin \( \delta_2 \) as illustrated in LXXXII.

\[
\begin{align*}
\text{LXXXVI} & : \\
\text{LXXXVII} & : \\
\text{LXXXVIII} & :
\end{align*}
\]

\[
\begin{align*}
\text{LXXXIX} & : \\
\text{(XC)} & : \\
\text{(XCI)} & :
\end{align*}
\]

The albomycins each contain one molecule of sulphur and alkaline hydrolysates were reported to give nearly quantitative yields of sulphate. Similarly, a fragment \( H_2 \) (LXXXV), derived from partial acid hydrolysis of albomycin, was reported to yield sulphate upon alkaline hydrolysis. No desulphurized reaction product could be isolated after treatment of albomycin with Raney nickel and, in an audacious proposal, the sulphur atom was thus assigned to a sulphone bridge connecting the pyrimidine moiety with a hydroxyl group of serine, giving rise to a sulphamate.

The structures of albomycins \( \delta_2, \delta_1 \), and \( \varepsilon \) proposed by Sorm and co-workers are depicted by formulae LXXXII–LXXXIV.

Although among the earliest antibiotics to be discovered, very little regarding the chemistry of grisein has been published. The antibiotic is produced by \textit{Streptomyces griseus} and was never prepared in pure form. Nevertheless, a comparison of albomycin and grisein in terms of biological properties and partition chromatographic behaviour revealed their great similarity and possible identity. These conclusions were supported by later studies, but, due to the lack of pure grisein, rigorous proof of identity with albomycin could never be established. A similar dilemma prevails for alveomycin, antibiotics A 17871, LA 5352 and LA 59371, and 10073 which are similar to and perhaps identical with albomycin.
Recently, we have isolated an antibiotic complex from the culture fluid of *Streptomyces griseus* var. X-2455 which closely resembled albomycin. Hydroiodic acid hydrolysis of this antibiotic complex, however, yielded serine and ornithine in a molar ratio of 1:3 and it was therefore concluded that this complex was different from albomycin. Ion exchange and partition chromatography resolved the complex into three antibiotics, designated Ro 5-2667, Ro 7-7730 and Ro 7-7731. The u.v.-spectra of these three antibiotics were qualitatively identical with those of albomycins δ₂, δ₁ and ε, although a quantitative comparison was not possible since absorbancies for albomycins were never reported.

Hydriodic acid hydrolysis of antibiotics Ro 5-2667, Ro 7-7730 and Ro 7-7731 confirmed the molar ratio of serine to ornithine as 1:3 in each and performic acid oxidations, followed by hydrochloric acid hydrolyses, indicated molar ratios of serine to glutamic acid of 1:3.

An *in vitro* comparison of Ro 5-2667, Ro 7-7730 and Ro 7-7731 with albomycin complex further confirmed similarity. Identity with albomycin was again suspected when it was observed that Ro 7-7731 could act as antibiotic, at 35°, against a *Serratia* sp. but antagonized the antibiotic activity of compounds Ro 5-2667 and Ro 7-7730 at 22°. With *Bacillus subtilis* as test organism this antagonism was clearly observed at 35°. A similar peculiarity of albomycin ε had previously been noted. Upon thin layer chromatographic comparison in several solvent systems albomycin δ₂ and Ro 5-2667, as well as albomycin δ₁ and Ro 7-7730, could not be differentiated.

To re-investigate published hydrolysis data, the albomycin complex† was separated in our laboratory into its components δ₂, δ₁ and ε and hydroiodic acid hydrolyses of albomycins δ₁ and δ₂ indeed revealed molar ratios of serine to ornithine of 1:3 in both preparations. Similarly, both albomycins yielded, after Fe(III) removal, performic acid oxidations and hydrochloric acid hydrolyses, serine and glutamic acid in molar ratios of 1:3. Identical n.m.r. spectra of iron-free preparations of the major antibiotics, albomycin δ₂ and Ro 5-2667, established definite proof of identity.

Iron-free antibiotic Ro 5-2667 yielded N⁵-hydroxy-L-ornithine and L-serine upon hydrochloric acid hydrolysis and catalytic hydrogenation of the hydrolysate afforded serine and ornithine in a molar ratio of 1:3. Periodate oxidation and hydroiodic acid hydrolysis gave acetic acid and ornithine, respectively, in equimolar amounts. Similarly, iron and sulphur were present in equimolar amounts and the molar ratio of acetic acid to iron was 3:1. The iron-binding centre is thus composed of three N⁵-acetyl-N⁵-hydroxyornithine moieties. The assignment of a trihydroxamate structure was compatible with the corresponding acid stability of the Fe(III) trihydroxamate complex, whereas n.m.r. spectra confirmed the presence of three N⁵-acetyl-N⁵-hydroxyornithine moieties and only one serine residue. The presence of an additional acetyl group was ruled out by n.m.r. spectroscopy, eliminating the possibility of an N⁴-acetylcytosine moiety, as proposed for albomycin δ₂ by Sorm and co-workers. A re-investigation of the u.v.

† Albomycin samples were obtained from the Soviet Union through the courtesy of Dr R. Morf and from the laboratories of Prof. Sorm.
spectra of compound XCl which, in part, were responsible for Šorm's proposed structure LXXXII and a consideration of dissociation data confirmed the disagreement between LXXXII and XCl. 5-Substituted pyrimidines, proposed by Poddubnaya, are ruled out on the basis of clearly discernible AB patterns in the n.m.r. spectra of Ro 5-2667, albomycin δ₂, Ro 7-7730 and Ro 7-7731, due to olefinic protons 5 and 6 of the pyrimidine moieties. The assignment of the sulphur atom proposed by Šorm and collaborators (LXXXII–LXXXV) appears to be incorrect in two respects. The recovery of sulphate from albomycin after alkaline hydrolysis could not be duplicated with antibiotic Ro 5-2667. In contrast, pyrolysis of antibiotic Ro 5-2667 at 250° liberated hydrogen sulphide, as did treatment with zinc and dilute hydrochloric acid. Secondly, the sulphur moiety cannot be attached to the pyrimidine part as depicted by Šorm and co-workers (LXXXII–LXXXV) in view of CD spectra of iron-free compounds Ro 5-2667, Ro 7-7730 and Ro 7-7731, which suggested the attachment of the pyrimidine moieties to asymmetric carbon atoms.

The many discrepancies observed between albomycin δ₂ and antibiotic Ro 5-2667 indicate the necessity for a critical reappraisal of the published structures for albomycins.

It was observed previously that similar iron-binding sites appertain to both ferrimycin and ferrimycin-antagonists, the ferrioxamines. Similarly, ferrimycin decomposes under mild conditions creating ferrimycin-antagonists, most probably with unchanged iron-binding centres. It appears, therefore, that effective sideromycin antagonism requires close chemical similarity of the iron-binding sites between both sideromycin and sideramine. In further agreement, ferrichrome, ferrichrysin and ferricrocin, distinguished by N⁵-acetyl-N⁵-hydroxyornithine moieties, resemble albomycin closely and antagonize the antibiotic activity of albomycin against Gram positive and Gram negative organisms more effectively than the more distant relatives LXXVII, LXXX and LXXXI. The iron-binding centres of ferrimycins and ferrichrome-type sideramines are quite different and, as a result, antagonism prevails only with Staph. aureus. Similar differences among the iron-binding centres exist between albomycin, with ferrichrome-like trihydroxamate arrangement, and ferrioxamines, hence, limited antagonism between albomycin and ferrioxamines is observed. Albomycin e and siderochrome Ro 7-7731 can act as antibiotics or albomycin δ antagonists. Clearly, this special behaviour is explained by a similarity between the sideramine and sideromycin component which is not limited to the iron-binding site but embraces the majority of the molecules.

3.1.3. Hydroxamic acid functions connected head to head via amide bonds and head to tail via ester bond

Coprogen. This compound is a metabolite of various fungi and acts as a growth factor for Pilobolus species. Structure elucidation was achieved nearly two decades after the discovery of this sideramine.

Coprogen is a neutral compound and contains L-N⁵-hydroxyornithine (XII) as the only amino acid and hydroxylamino moiety. A ferrirubin-type trihydroxamate grouping was suspected because of spectroscopic similarity with compounds LXXVII, LXXX and LXXXI and the liberation of 5-
hydroxy-3-methylpent-2-enoic acid upon periodic acid oxidation of deferricoprogen. Acetic acid was obtained upon acid hydrolysis of coprogen and n.m.r. spectra suggested its origin in an N-acetyl function.

Three trans-fusarinine units and one acetic acid molecule were indicated as the only components of coprogen based on elemental analysis and n.m.r. spectra. The ester bond (1730 cm\(^{-1}\)) was ammonolized resulting in the addition of one mole of ammonia; removal of iron from the ammonolysis product gave dimeric acid, XLIII. Two free hydroxyl groupings were suggested by acetylation studies leading to structure XCII for coprogen.

Coprogen B is desacetyl coprogen and is produced by a variety of fungi. The antibiotic activity of ferrimycin A and albomycin against Staph. aureus is antagonized to the same extent by coprogen, coprogen B, fusigen (XCIII) and dimeric acid. Possibly related to coprogen and ferrichromes is Terregens Factor, a bacterial growth factor produced by Arthrobacter pascens.

3.2. Compounds with three hydroxamic acid functions in a cyclic skeleton

3.2.1. Hydroxamic acid functions connected head to tail via ester bonds

Fusigen. A close relative of fusararine B, fusigen was isolated from culture fluids of fungi. As a trimer of cis-fusararine, structure XCIII was assigned to fusigen based on physico-chemical data and degradation products. Fusigen exhibits growth-factor activity and is probably identical with fusararine C. The terms fusigen B and fusararine B are synonymous as are fusigen C and fusararine A.
3.2.2. Hydroxamic acid functions connected head to tail via amide bonds

Nocardamine (Ferrioxamine E). This compound was originally described\textsuperscript{124} as a narrow-spectrum antibiotic with activity against mycobacteria. Hydrolysis with hydrochloric acid yielded succinic acid and a hitherto unknown compound\textsuperscript{125}. Some ten years later\textsuperscript{81} 1-amino-5-hydroxylaminopentane (LXVII) was isolated as a hydrolysis product of ferrioxamines and ferrimycins and it was not until then that the structure of the new nocardamine-derived hydrolysis product could be correctly identified as LXVII\textsuperscript{82}.

The representation of nocardamine as a cyclic, eleven-membered monohydroxamic acid involving one molecule each of succinic acid and 1-amino-5-hydroxylaminopentane\textsuperscript{82} was untenable on the basis of polarographic data\textsuperscript{126} which favoured the trihydroxamate structure XCIV. This assignment
was supported by molecular weight determinations and later by the availability of stability constants of various Fe(III) complexes. Whereas ferrioxamine B is 200 times more stable than the tris-acetylhydroxamato-complex, nocardamine is 16000 times more stable. The relative difference in chelate effects of ferrioxamine B and nocardamine is explained by linearity and cyclicity, respectively, of the molecules, giving rise to greatly different entropy changes upon complex formation.

**Ferrioxamine D₂.** Ferrioxamines B and A₁ are in the same homologous relationship as are nocardamine and ferrioxamine D₂. Similar to previous examples, structure XCV was deduced for ferrioxamine D₂ by titration data, hydrochloric acid hydrolysis and n.m.r. studies.

\[ \text{Ferrioxamine D₂ (XCV)} \]

### 4. UNCLASSIFIED COMPOUNDS CONTAINING HYDROXAMATE FUNCTIONS

Succinimycin, produced by *Streptomyces olivochromogenes*, is active against certain Gram positive bacteria and shows cross-resistance with grisein. Ammonia, methylamine, succinic acid and LXVIII are among the products of hydrolysis with dilute alkali. Similarly, danomycin, a metabolite of *Streptomyces albadancus*, is active preferentially against Gram positive bacteria and, upon acid hydrolysis, yields acetic acid, succinic acid, LXVIII and 17 amino acids. Like succinimycin, danomycin is cross-resistant with grisein.

Little is known about the toxic gluconimycin which is elaborated by *Streptomyces AS9* and shows activity against Gram positive and Gram negative bacteria and fungi. Antibiotic ASK-753, derived from *Streptomyces AS-K-753*, is active mainly against Gram positive bacteria and to a lesser extent against Gram negative organisms and non-filamentous fungi. The antibacterial activity is not antagonized by ferrioxamine B. Antibiotics 22765 and 22931 have received little attention.

Hydroxamic acid functions are suspected in conalbumin of egg white, in siderophylin, the iron-binding β-pseudoglobulin of human plasma.
HUBERT MAEHR

and in ferritin\textsuperscript{133}, a brown pigment occurring in spleen, bone marrow and liver.

Schizokinen, an iron transport factor produced by \textit{Bacillus megaterium} Texas, is responsible for the initiation and maintenance of exponential cell division of certain microorganisms\textsuperscript{134a}. Schizokinen antagonized ferrimycin-A activity against three \textit{Bacillus} species; it was isolated as an Fe(III) complex. A schematic model of the conjugal transfer of R factors is depicted in

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